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Effects of Arginine Supplementation on Organ Development, Egg Quality, Serum Biochemical parameters, and Immune Status of Laying Hens

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■Keywords

Arginine, clinical blood parameters, immune status, laying hens, organs development.

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ABSTRACT

This experiment was conducted to study the effects of arginine supplementation on organ development, egg quality, blood parameters, and immune status of laying hens. A total of 360 25-week-old brown Leghorn laying hens were randomly divided into three groups with six replicates of 20 birds each and fed diets supplemented with 0, 8.5, or 17 mg of L-arginine/kg for 42 days. Results showed that the weight of proventriculus and duodenum in the treatment supplemented with 17 mg/kg L-arginine was heavier than that of 0 mg L-arginine/kg treatment ($p<0.05$). The weight of oviduct in the treatments supplemented with 17 mg/kg L-arginine was smaller than that of 0 and 8.5 mg L-arginine/kg treatment ($p<0.05$), and the small yellow follicle amount in the treatment supplemented with 17 mg/kg L-arginine was less than that of 0 mg L-arginine/kg treatment ($p<0.05$), while its yolk color was deeper than that of 0 mg L-arginine/kg treatment ($p<0.05$), and the IgY content showed the same tendency. Total cholesterol and triglyceride levels in the treatment supplemented with 8.5 mg/kg L-arginine were lower than that of 0 mg L-arginine/kg treatment ($p<0.05$). The concentration of IL-2 in the treatment supplemented with 17 mg/kg L-arginine were more than that of 0 mg L-arginine/kg treatment ($p<0.05$). The findings of this study show that 17 mg/kg L-arginine supplementation has beneficial effects onlayers' immune status and yolk IgY content, as well as on proventriculus and duodenum weight while no adverse effects were observed on laying performance, egg quality, or blood parameters.

INTRODUCTION

Arginine (Arg), a ubiquitous basic amino acid, is necessary for maintenance, growth, reproduction, and immunity (Wu, 2009). Poultry are not able to synthesize arginine themselves, and therefore completely depend on dietary arginine to meet their arginine needs for protein synthesis and other functions (D'Mello & Lewis, 1971). In addition, arginine becomes the most important amino acid when lysine is adequate in diet. Arginine plays a vital role in the modulation of protective immune response (Tayade *et al*., 2006), and it is an important substrate for the immune system (Amato &Humphrey, 2010). Cengiz *et al*. (2010) found that Arg supplementation reduced proteinemia, and modified erythrocyte characteristics, increasing their mean volume and reducing mean corpuscular hemoglobin load.

In 2010, Khajali & Wideman considered that the arginine recommendations of the NRC might not be adequate to support maximal growth and arginine-depleting immune responses, and to prevent the onset of pulmonary hypertension in broilers reared under rigorous environmental conditions. Perez-Carbajal *et al*. (2010) found that arginine fed at levels higher than those recommended by the NRC

could play a complementary role in the innate and humoral immune responses, potentially enhancing the immune response to field infections.

Although there are many studies in literature on arginine in broilers, few studies were found in laying hens. Therefore, it makes sense to study the effect of arginine on the performance and immune status of laying hens. The objective of the present study was to establish the effect of arginine on laying performance, egg quality, organ development, blood parameters, and immune status of laying hens. http://ps.fass.org/content/89/9/1870 - aff-3#aff-3

MATERIALS AND METHODS

Experiment Design

The experiment was carried out in the facilities of Jinghu Yongxiang breeding hens Co., LTD,. Huaian, Jiangsu Province, China. Three hundred and sixty 25-week-old brown *Leghorn* laying hens were randomly divided into three treatments with six replicates of 20 birds each (120 laying hens per group). The birds were fed diets supplemented with 0 mg/kg, 8.5 mg/kg, or 17 mg/kg of L-arginine for 42 days. Hens were maintained in a layer house with tiled roof at a density of two birds per battery cage (50×50 cm). The photoperiod was set at 16L:8D throughout the study. Housing temperature and relative humidity, controlled by automatic system, were kept at 25- 29 °C and 65-75 %, respectively. Water and feed were provided *ad libitum* during the study in automatic trough drinkers and feeders, respectively. Egg production was recorded daily, and the laying ratio was calculated by using whole egg production dividing feeding days.

Table 1 presents the composition of the experimental diets. All animal handing protocols were approved by of the Animal Care and Use Committee of the University.

Table 1 – Composition and nutrient levels of the diet (air-dried basis) %

Ingredients	Content	Nutrient levels[2]	Content
Corn	62.5	ME/(MJ/kg)	11.91
Soybean meal	16.2	Crude Protein	16.02
Corn gluten meal	6.0	Calcium	3.75
Limestone	8.0	Available Phosphorus	0.40
Soybean oil	2.3	Arginine	0.87
Premix[1]	5.0		
Total	100.0		

[1]The premix provided the following per kg of the diet, Vit. A 12,500 IU, Vit. $D_3$4,125 IU, Vit. E 15 IU, Vit. K 2 mg, thiamine 1 mg, riboflavin 8.5 mg, calcium pantothenate 50 mg, nicotinic acid 32.5 mg, pyridoxine 8 mg, VB_{12} 5 mg, biotin 2 mg, Fe 60 mg, Cu 8 mg, Zn 66 mg, Mn 65 mg, Se 0.3 mg, I 1 mg.

[2]Nutrient levels were all measured values.

Blood Parameters

At the end of the 42-day feeding period, blood samples (2.5 mL) were collected from the wing vein of 54 birds (three birds per replicate, 18 birds per treatment) for blood chemistry. Blood samples centrifuged at 3500g for 10 min, and the following parameters were determined in the serum: total protein (TP), albumin (ALB), aspartate aminotransferase (AST), total cholesterol, and triglyceride levels. All parameters were measured using a biochemical analyzer (UniCel DxC 800 Synchron, Beckman coulter, USA).

Organ Development

After the collection of blood samples, all the birds were killed by exsanguination. The following organs were weighed (paired organs were weighed together): proventriculus, gizzard, heart, liver, spleen, duodenum, jejunum, ileum, cecum, rectum, oviduct, ovarium, and follicle. In addition, oviduct length, and number of preovulatory follicles amount, of small yellow follicles, and of large white follicle amounts were also determined.

Egg Quality

Eighteen eggs per treatment (3 eggs per replicate) were collected at the end of the study to determine egg quality parameters. Egg shape index was calculated according to the formula: shape index = height/width (Anderson *et al*., 2004). Eggs were weighed and the shell strength of intact eggs was measured with an Instron testing machine (TSS, York, UK.). A constantly increasing load was applied to an egg lying lengthways until it broke. The applied load at the time of breakage is the measured shell strength. Eggs were cracked onto a flat surface, and the height of the albumen was measured using a digital albumen height gauge (TSS, York, UK). The measurement was taken at three points of the thick and flat albumen in the 1-cm diameter area around the egg yolk, forming an equilateral triangle. After separated from the albumen, the yolk was weighed. Albumen mass was calculated as the difference between egg weight and the sum of yolk and eggshell weights, and expressed as a percentage of egg weight. Yolk color was evaluated with DSM Yolk Color Fan, which included 15 colorimetric blades according to their intensity of yellow (QCC-System, TSS), and expressed in grades. Eggshell thickness was the mean value of the measurements made at three different locations (air cell, equator, and sharp end), and determined in the fresh shell without membranes by a digital micrometer (TSS).

In addition, all the egg yolks were frozen at -20°C for IgY analysis. The concentration of yolk IgY was measured according to a commercial Chicken IgY ELISA Quantitation Kit provided by Nanjing jiancheng Co. (Nanjing, China).

Immune Status

The blood concentrations of interleukin-2 (IL-2) and interferon-γ (IFN-γ) were measured using an enzyme-linked immuno sorbent assay-based method according to Dalloul *et al*. (2003).

Statistical Analysis

All data were subjected to repeated measure analysis, using the mean of each replicate as the experimental unit. All data were analyzed using SPSS software (SPSS 17.0 for windows). One-way analysis of variance, followed by a Duncan's multiple comparison test, were applied to separate different means among treatments. Data were assumed to be statistically significant when $p<0.05$.

RESULTS

Blood Parameters

Total serum cholesterol and triglyceride levels of the hens in the treatment supplemented with 8.5 mg/kg L-arginine were lower than those of hens in the 0 mg L-arginine/kg treatment ($p<0.05$, Table 2). There were no effects of dietary arginine supplementation on serum TP, ALB, or AST levels ($p>0.05$).

Table 2 – Effects of arginine on blood parameters

Items	Arginine (mg/kg)		
	0	8.5	17
TP (g/L)	35.14±1.21	34.84±0.94	34.90±0.52
ALB (g/L)	21.98±0.40	21.97±0.33	21.96±1.17
AST (U/L)	208.27±10.31	213.20±9.19	202.44±5.86
Total cholesterol/ (mmol/L)	3.82^{a}±0.29	3.12^{b}±0.21	3.26^{ab}±0.15
Triglyceride/ (mmol/L)	11.35^{a}±0.80	8.90^{b}±0.86	10.07^{ab}±0.69

^{a,b}Mean ± SE with different superscripts within a column are significantly different ($p<0.05$).

Organ Development

Proventriculus weight of the hens in the treatment supplemented with 17 mg/kg L-arginine was heavier compared with the 0 mg L-arginine/kg treatment ($p<0.05$, Table 3). Duodenum weight of the hens in the treatments supplemented with 8.5 and 17 mg/kg L-arginine was heavier than that of hens fed 0 mg L-arginine/kg ($p<0.05$). There were no differences in the weight of body, gizzard, heart, liver, spleen, jejunum, ileum, cecum and rectum among the treatments ($p>0.05$).

Table 3 – Effects of arginine on digestive tract development

Items	Arginine (mg/kg)		
	0	8.5	17
Proventriculus weight (g)	5.46^{a}±0.18	5.74^{ab}±0.14	6.23^{b}±0.27
Gizzard weight (g)	22.69±0.61	22.72±0.49	23.85±0.48
Heart weight (g)	6.10±0.16	5.85±0.20	5.91±0.18
Liver weight (g)	26.73±0.99	27.62±0.99	28.13±0.77
Spleen weight (g)	1.62±0.10	1.77±0.11	1.90±0.13
Duodenum weight (g)	6.72^{a}±0.20	7.41^{b}±0.26	7.52^{b}±0.23
Jejunum weight (g)	13.26±0.44	13.05±0.54	13.71±0.54
Ileum weight (g)	9.28±0.28	9.35±0.32	9.02±0.35
Cecum weight (g)	5.38±0.15	5.59±0.21	5.65±0.16
Rectum weight (g)	3.28±0.17	3.19±0.15	3.44±0.16

^{a,b}Mean ± SE with different superscripts within a column are significantly different ($p<0.05$).

Reproductive Organ Development

Oviduct weight of the hens in the treatment supplemented with17mg/kg L-arginine was lighter than that of those fed 0 and 8.5 mg L-arginine/kg treatment ($p<0.05$, Table 4). The number of small yellow follicles of the hens supplemented with 17 mg/kg L-arginine was lower than that of the hens fed 0 mg L-arginine/kg ($p<0.05$). There were no differences among treatments in oviduct length, weights of ovary and follicles, preovulatory follicle number and number of big white follicles $p>0.05$).

Table 4 – Effects of arginine on reproductive organ development

Items	Arginine (mg/kg)		
	0	8.5	17
Oviduct weight (g)	54.06^{a}±1.84	54.89^{a}±2.11	47.86^{b}±1.27
Oviduct length (cm)	56.56±1.09	58.90±1.29	56.15±0.90
Ovarium weight (g)	4.33±0.22	4.52±0.19	4.78±0.25
Follicle weight (g)	27.42±1.35	26.57±1.77	27.95±1.75
Number of follicles	18.69±1.58	17.57±1.21	17.58±1.23
Number of preovulatory follicles	4.00±0.34	4.00±0.21	4.12±0.15
Number of small yellow follicles	6.31^{a}±0.86	5.14^{ab}±0.50	4.06^{b}±0.56
Number of large white follicles	8.38±1.06	8.43±0.95	9.40±0.94

^{a,b}Mean ± SE with different superscripts within a column are significantly different ($p<0.05$).

Egg Quality

The yolk color of the eggs of the hens supplemented with 17 mg/kg L-arginine was deeper than those of hens fed 0 mg L-arginine/kg ($p<0.05$, Table 5). The laying ratio and the other egg quality parameters measured were unaffected by dietary arginine ($p>0.05$).

Table 5 – Effects of arginine on performance and egg quality

Items	Arginine (mg/kg)		
	0	8.5	17
Laying ratio (%)	91.38±0.86	90.51±0.20	89.61±0.47
Average egg weight (g)	53.44±0.13	52.86±0.30	52.78±0.43
Shape index of egg	1.28±0.01	1.27±0.01	1.29±0.01
Eggshell strength (kg/cm²)	4739±136	4821±174	4654±196
Eggshell thickness (mm)	0.32±0.01	0.31±0.00	0.30±0.01
Albumen height (mm)	6.11±0.16	6.29±0.17	6.53±0.24
Eggshell weight (g)	7.08±0.15	6.81±0.12	6.92±0.17
Yolk weight (g)	12.12±0.19	12.71±0.29	12.17±0.25
Yolk color	8.19 ^{a}±0.19	8.63 ^{ab}±0.15	8.94 ^{b}±0.17

^{a,b}Mean ± SE with different superscripts within a column are significantly different ($p<0.05$).

Immune Status

The IgY content change showed the same tendency, and the IgY content of the eggs of the hens supplemented with 17 mg/kg L-arginine was 62.8% higher compared with those laid by the hens in the 0 mg L-arginine/kg treatment. The concentration of IL-2 in the eggs of the hens fed 17 mg/kg L-arginine were 41.8% higher compared with the eggs of the hens in the 0 mg L-arginine/kg treatment ($p<0.05$, Table 6).

Table 6 – Effects of arginine on immune status

Items	Arginine (mg/kg)		
	0	8.5	17
IgY content (μg)	90.92 ^{a}±10.53	104.42 ^{ab}±8.71	148.02 ^{b}±35.14
IL-2 (ng/L)	3.85 ^{a}±0.37	4.31 ^{ab}±0.38	5.46 ^{b}±0.69
IFN-γ (ng/L)	60.81±6.49	68.70±6.86	79.02±9.76

^{a,b}Mean ± SE with different superscripts within a column are significantly different ($p<0.05$).

DISCUSSION

Blood Parameter

In the present experiment, total cholesterol and triglyceride serum levels in the hens supplemented with 8.5 mg/kg L-arginine was lower than that of 0 mg L-arginine/kg treatment, which was in accordance with many previous results (Fouad *et al*., 2013; Ueda *et al*., 1995; Al-Daraji *et al*., 2012). Fouad *et al*. (2013) and Ueda *et al*. (1995) found that dietary arginine supplementation significantly decreased blood serum cholesterol. Al-Daraji *et al*. (2012) found that the dietary arginine supplementation significantly decreased blood total lipids and triglyceride concentrations.

Organ Development

Arginine supplementation had a weak influence on organ weight. Cengiz & Kucukersan (2010) did not find any significant differences in the carcass traits of broilers fed diets supplemented with arginine or not. Leitgeb *et al*. (2004) also verified that broiler organ weight, carcass chemical composition, and meat quality (tenderness, juiciness and taste) were not influenced by the dietary supplementation of with different levels of arginine. Jahanian (2009) found that dietary arginine deficiency affected the thymus and the spleen of broilers. However, all the above studies were performed with broilers, and few studies concerning on arginine in laying hens were published. In this study, there were no significant differences in viscera weights except for the proventriculus and duodenum. How these two organs were stimulated to develop much heavier? Was arginine the reason? Further research is needed to elucidate the mechanisms of this phenomenon.

Reproductive Organ Development and Egg Quality

Arginine supplementation reduced oviduct weight and the number of small yellow follicles, while it had no significant influence on the other reproductive organs. Even though the number of small yellow follicles in the treatment supplemented with 17 mg/kg L-arginine was lower than with that in the control group, the number of preovulatory follicles was not different. This may explained by a higher apoptosis rated of small yellow follicles in hens supplemented with 17 mg/kg L-arginine compared with the control group.

The yolk color and IgY content of the eggs from hens supplemented with 17 mg/kg L-arginine increased compared with that in the control group, which may explained by the promotion of lutein and IgY deposition by dietary arginine. In this experiment, daily feed intake and laying ratio were120 g and 90%, respectively, and consequently, 2.04 mg dietary arginine supplementation resulted in about 57 μg IgY in the egg. Therefore, it was an effective method to produce a functional egg containing large quantities of IgY, which could be used to prevent diseases.

Immune Status

In this experiment, dietary arginine supplementation increased the serum concentration of IL-2. Arginine supplementation may enhance the immune status of animals. Cengiz & Kucukersan (2010) found that arginine promoted non-specific immunity with monocyte expansion. Arginine supplementation stimulates the functional activities of different cell types, including natural-killer cells, and the systemic immune response against IBDV in chickens (Tayade *et al*., 2006)

and anti-BSA (bovine serum albumin) IgM levels in lying hens (Deng *et al*., 2005). In addition, arginine may improve local wound healing by decreasing the inflammatory response at the wound site (Angele *et al*., 2002).

The duration and amount of arginine supplementation may influence immune status. Deng *et al*. (2005) found that short-term supplementary arginine had minimal effects on immunity, but some enhancement of SRBC antibody responses in later stages of growth was observed with previous arginine administration. In addition, a study found minimal effects of arginine supplementation on broiler growth and immune parameters (Kidd *et al*., 2001), possibly because the levels of arginine supplementation were low. On the other hand, the proportion of heterophils in peripheral blood was reduced in broiler chicks fed Arg-deficient diets (Jahanian, 2009). Moreover, a decrease in dietary crude protein and Arg levels diminished the antibody production response to Newcastle disease virus (Jahanian, 2009), suggesting that a minimal level of arginine needs to be supplied in poultry diets.

In conclusion, the findings of this study show that the supplementation of 17 mg/kg L-arginine to layer diets benefits their immune status and increases yolk IgY content, as well as promotes heavier proventriculus and duodenum, while no adverse effects are observed on laying performance, egg quality, or blood parameters.

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